UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: 08/08/2018

SUBJECT: **Strychnine:** Summary of Hazard and Science Policy Council (HASPOC) Meeting of December 3, 2015: Recommendation on the requirement for a non-food use database of toxicity studies.

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TO: Jaime D'Agostino, Toxicologist
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MEETING ATTENDEES

HASPOC Members: Anna Lowit, Elissa Reaves, Elizabeth Mendez, Michael Metzger, P.V. Shah, Ray Kent, Matt Lloyd, Jeff Dawson, Jonathan Leshin, Uma Habiba, Sarah Gallagher

Presenters: Jaime D'Agostino and Matthew Crowley

Other Attendees: Don Wilbur, Yung Yang, Vincent Chen, Laura Nollen, Chris Schlosser, Bridget Boboweic, Anwar Dunbar, Donna Davis, Matt Crowley, Chris Olinger, Christina Swartz

I. PURPOSE OF MEETING

Risk Assessment Branch II (RAB II) is currently preparing a scoping document for the rodenticide strychnine as part of registration review. Based on the current toxicology data requirements, the entire non-food use toxicity database (see list below) is required due to potential for exposure to occupational handlers. However, the Hazard and Science Policy Council (HASPOC) met on December 3, 2015 to discuss the need for these studies for strychnine.

Outstanding studies for strychnine:

- 870.3200, 90-Day dermal
- 870.3465, 90-Day inhalation- rat (conditionally required; exposure pattern indicates a study would be required)
- 870.3700a, Prenatal developmental study in rat
- 870.3700b, Prenatal developmental study in rabbit
- 870.3800, Reproduction and fertility effects
- 870.5100, Bacterial reverse mutation assay
- 870.5300 and 870.5375, *In vitro* mammalian cell assay
- 870.5385 and 870.5395, *In vivo* cytogenetics
- 870.6200a, Acute neurotoxicity
- 870.6200b, Subchronic neurotoxicity
- 870.7800, Immunotoxicity

II. SUMMARY OF USE PROFILE & CURRENT RISK ASSESSMENT

Strychnine is a rodenticide that was first registered in the U.S. in 1947. Strychnine is a terpene indole alkaloid which acts as a convulsant by antagonizing glycine receptors. Glycine is an important inhibitory transmitter to motor neurons. Strychnine was also used as a therapeutic agent in humans between the 16th and mid-20th century but no longer has any medical uses. Strychnine is banned in most European countries. It is registered in the U.S. for underground use to control pocket gophers only, except for a special local need (SLN) registration in Nevada for underground control of yellow-bellied marmots and ground squirrels in addition to pocket gophers.

Strychnine is formulated as a 0.5% grain-based bait or granule. The SLN registration is a 3.2% paste restricted use product (RUP). The products are specified for use on pastures, rangeland, orchards, agricultural cropland, non-agricultural uncultivated areas, irrigation ditches/canals, and residential lawns. Some but not all products are restricted-use. Based on label descriptions, application of granule products can be by spoons/cups, hand probe dispensers, mechanical burrow builders, and hand dispersal. The label for the SLN paste product instructs users to mix the paste with cut vegetables in a large plastic bag and form baits which are then placed in the burrows by hand or with a long-handled spoon. As a result of the registered uses, there is a potential for short-term occupational exposure associated with handler activities; intermediate-term and chronic exposures are not expected. Additionally, no post-application assessments are needed and incidental oral or episodic granular exposure to children are not expected due to the underground nature of the uses. Personal protective equipment (PPE) requirements vary on product labels. Some only require baseline work attire (long sleeve shirt, long pants, shoes/socks) with cotton gloves, while others require chemical/water-resistant gloves and dust/mist filtering respirators.

In the most recent risk assessment [HED chapter of the Reregistration Eligibility Decision (RED) Document, J. Smith, 1996], the toxicological database was considered adequate for the current non-food uses which included only acute lethality studies. In addition, at that time, three data gaps were identified as confirmatory data: an acute inhalation LD_{50} study, a dermal sensitization study in guinea pigs, and a 21-day dermal toxicity study in the rat. Of the studies requested, only acute inhalation LD_{50} studies (with a 0.5% and a 1.8% formulation) and dermal sensitization studies in guinea pigs (with a 0.5% formulation and the technical) were submitted.

Since strychnine is registered as a non-food use, no dietary risk assessment was conducted. Furthermore, strychnine residues in water are expected to be negligible and therefore, significant drinking water exposure is not anticipated. The 1996 RED did not identify short-, intermediate-, or long-term/chronic toxicological endpoints for strychnine and did not quantitatively assess risk for residential or occupational handlers. Still, the evaluation concluded that there were possible risks of concern for applicators based on: 1) the high acute toxicity through oral (Toxicity Category I), ocular (Toxicity Category I), and presumably inhalation (was rated as Toxicity Category I the absence of the acute inhalation LC_{50} study); 2) incidence data for domestic pets indicate that strychnine exposure was lethal in 19% of cases, primarily in dogs, which supports that low levels of ingestion can result in significant health effects; 3) the number and severity of poisoning incidents reflected in epidemiological data; 4) the potential for exposure to both occupational and residential handlers; and 5) the absence of exposure data for most scenarios. Based on this, a number of risk mitigation recommendations were made to limit the homeowner use of strychnine (e.g., no more than 0.5% active ingredient) and also to mandate PPE for occupational uses (e.g., chemical-resistant gloves and respirators for certain formulations). During registration review, HED anticipates conducting a quantitative occupational inhalation exposure assessment. No other quantitative exposure assessments are anticipated; there is no anticipated exposure to children, there is no dietary exposure, and the lack of dermal toxicity following acute exposure up to 2,000 mg/kg, despite potent acute toxicity following other routes of exposure, supports that a quantitative occupational dermal assessment is not needed (please see the toxicity data below for a more details). Furthermore, there is no anticipated post-application exposure due to the underground nature of the applications and a residential handler

assessment will not be conducted because HED has determined that these products are not for homeowner use because product labels require specific clothing (e.g., long sleeve shirt/long pants) and/or use of personal protective equipment (PPE).

Recently, the United States Department of Agriculture (USDA) Forest Service completed a human health and ecological risk assessment for strychnine (P. Durkin, 2010). The risk assessment took into account a number of studies in the literature regarding exposure to experimental animals as well as accidental and suicidal exposures to humans. The conclusion of the USDA Forestry Service was that under normal and anticipated circumstances, the use of strychnine in below-ground applications should pose minimal risk to workers and members of the general public. In addition, HED's Toxicology and Epidemiology Branch has conducted a more recent review of human incidents and epidemiology data (S. Recore, D430176, 2015) for the period of 1998-2011, which is following implementation of the mitigation recommended in the RED above. Based on the low frequency and severity of the incidents reported in IDS and SENSOR-Pesticides, there does not appear to be a concern that warrants further investigation.

III. TOXICITY DATA FOR STRYCHNINE

While no mechanism of action (MOA) studies were submitted for strychnine, the mechanism for mammalian toxicity is well known for this compound based on its previous medical uses and a significant body of literature. Please see the U.S. Department of Agriculture (USDA) Forestry Services human health and ecological risk assessment for strychnine (P. Durkin, 2010) for a comprehensive list of references for the proposed mechanism of action of strychnine. The following is a brief summary based on the information presented in that document. Strychnine acts as an antagonist for glycine receptors, which is a ligand-gated chloride channel in neurons. Glycine is a neurotransmitter critical to the normal function of the nervous system. When glycine binds to the glycine receptor on neurons it increases the flow of chloride ions into motor neurons which results in inhibition of motor neuron activity. In contrast, binding of strychnine to chloride channels does not result in an increased flow of chloride ions and interferes with the normal inhibitory function of glycine. The net result is that strychnine leads to hyper excitability by preventing glycine from inhibiting the activity of the motor neurons. At very low doses, strychnine acts as a stimulant or performance enhancer, however at higher doses this leads to spastic muscle contractions, resulting in death by asphyxiation.

No guideline metabolism studies were submitted for strychnine. However, the toxicokinetics of strychnine have also been well-characterized in the literature based on its medical uses, accidental or suicidal human poisonings, and experimental studies in mammals. The following is a brief summary based primarily on the USDA Forestry Services review of the literature (P. Durkin, 2010). Strychnine is rapidly absorbed following oral administration. Based on human suicidal ingestions, peak concentrations of strychnine in plasma were achieved within 2-4 hours (Edmunds et al., 1986 and Palatnick et al., 1997). This is in line with the available guideline acute oral LD_{50} study with technical strychnine (MRID 40908901/41210701), where death was observed within 1 hour of dosing, and a literature study in rats (Seidl and Zbinden, 1982), where increased muscle tone and slight tremor were seen within 10-20 minutes and gradually subsided during the following hour. Strychnine is rapidly detoxified in the liver by cytochrome P450s, with the primary metabolite being a 21, 22-epoxide which is further broken down into two

dihydroxy metabolites. Strychnine appears to be rapidly cleared from both plasma and the body. In humans following suicidal ingestion, the plasma half-life of strychnine has been estimated at between 10 (Edmunds et al., 1986) and 15.9 (Palatnick et al., 1997) hours. Elimination of 0.5 mg/kg radiolabeled strychnine was measured in rats following subcutaneous injection (Oguri et al., 1989). After 24 hours, 60% was eliminated in the feces and 30% was eliminated in the urine indicating that a single dose of strychnine was almost completely eliminated in a day.

The following hazard characterization of strychnine is based off a combination of the guideline data received (acute six pack studies) and literature studies as summarized in the USDA Forestry Service risk assessment. Strychnine is highly acutely toxic following exposure by the oral route. In the guideline oral acute lethality study (MRID 40908901/41210701), death occurred within one hour of exposure with LD_{50} values of 6.4 mg/kg and 2.2 mg/kg in males and females, respectively. Of note was that females were more sensitive to the effects of strychnine than males. This is consistently observed across multiple literature studies in rats, although the effect is much less pronounced in mice. Strychnine is also known to be acutely toxic to humans. Based on a number of suicidal and accidental poisonings, the fatal dose is estimated to be between 1.4 and 80 mg/kg (P. Durkin, 2010, Table 6). The lowest lethal doses in humans are very similar to the LD_{50} values estimated in rats.

Strychnine is also highly acutely toxic following ocular and inhalation exposure. In the guideline eye irritation study with technical strychnine (MRID 40908904/41210704), acute ocular exposure resulted in slight irritation and mortality in 4 out of 6 rabbits at a dose equivalent to 52 mg/kg. In an acute inhalation study with a 1.8% formulation (MRID 44719701), female rats were observed with tremors and mortality at a dose of 2.13 mg/L (~ equivalent to an oral dose of 370.6 mg/kg).

In contrast, strychnine does not appear to be very acutely toxic by the dermal route. No irritation, mortality, or signs of toxicity were observed in the acute rabbit dermal lethality study with technical strychnine (MRID 40908902/41210702) up to a dose of 2000 mg/kg. This is likely due to a very low dermal absorption of strychnine. While no direct measurement of the dermal absorption for strychnine is available, the low dermal absorption of this chemical is supported by the National Institute for Occupational Health and Safety (NIOSH) finite dose skin permeation calculator (http://www.cdc.gov/niosh/topics/skin/finiteSkinPermCalc.html) which predicts a chemical's dermal absorption based on its physical chemistry parameters. Using the calculator for strychnine results in a predicted dermal absorption value of 0.25% over an 8-hour period with a dermal load of 1 $\mu g/cm^2$ (see Appendix for physical chemical properties used in this calculation). The Finite Dose Skin Permeation Calculator was developed through support of a Center of Disease Control/National Institute for Occupational Safety and Health (CDC/NIOSH) grant, and provides an estimation of fluxes, skin concentrations, and amounts absorbed from any size dose applied to partially or fully hydrated skin, using the physicochemical properties of the test compound and defined exposure parameters (Kasting, G.B. 2006; Wang, T.F. 2007; Kasting, G.B. 2008; Miller, M.A. 2010). Currently, OPP does not rely on (Q)SAR modeling alone to derive a Dermal Absorption Factor (DAF) for risk assessment. However, estimates from the Finite Dose Skin Permeation Calculator may be used with read across data to support a weight of the evidence evaluation.

Guideline data for repeat dosing of experimental mammals were not submitted and there is limited data on the repeat dose toxicity of strychnine in the literature due to the potent acute toxicity observed. For example, one study in the literature set out to determine a maximally tolerated dose of strychnine in rats following gavage dosing (Seidl and Zbinden, 1982). Consistent with the LD_{50} values determined in the guideline acute lethality study, some number of the rats in each sex died at these doses showing tonic muscle contractions and respiratory paralysis. The animals that survived showed increased muscle tone or a slight tremor 10 to 20 minutes following dosing that resolved within 60 minutes.

As indicated earlier, strychnine was used medically in humans for some time. Based on the literature, therapeutic doses range between 0.02 and 0.1 mg/kg (as reviewed in P. Durkin, 2010), which is at least an order of magnitude lower than the lowest reported lethal dose in humans (1.4 mg/kg). However, the long-term observations of patients undergoing strychnine therapy is limited in number and lacking in robustness. At best, the therapeutic doses suggest that any rapid onset of severe acute toxicity at these doses is not likely.

No guideline reproduction or developmental studies are available for strychnine and the data in experimental mammals in the literature are quite limited. There is one developmental study (Garcia-Alcocer et al., 2005) which links strychnine exposure to rats with neural tube defects at doses (5 and 8 mg/kg) that are above the LD_{50s} measured in the guideline acute oral lethality study. However, the reporting of the data is incomplete and the fact that the effect is observed at doses that are acutely toxic limits the usefulness of this study.

While no reproduction studies were available in the literature, a number of literature studies have attempted to address age differences in susceptibility in rats. For example, one study looked at 3-, 4-, 5- week and 6-month old rats and found that 5-week old rats were more sensitive than 6-month old rats by a factor of about 2, while 3-week old rats were more sensitive than 6-month old rats by a factor of about 4 (Davis and Yeh, 1969). The estimates of sensitivity were based on the dose of strychnine that resulted in 50% of the animals displaying clinical signs. Similar levels of sensitivity were reported by other authors as well when comparing similar age groups. Of note, another study compared neonatal rats (3-days old) to young rats (up to 25-days old) and found no difference in susceptibility (Kubova and Mares, 1995). Similar to the differences in sex sensitivity identified in rats, it appears the age-related sensitivity is less pronounced in mice (Lamanna and Hart, 1968).

IV. STUDY WAIVER REQUESTS

a. Determination of an Appropriate Endpoint to Evaluate the Need for Additional Studies

As described previously, no quantitative residential or occupational risk assessment was conducted during registration due to the lack of short-, intermediate- or long-term endpoints; the only toxicological data submitted were the acute lethality studies for the technical and formulations. In order to assess whether additional data are required for strychnine, HED performed screening level margin of exposure (MOE) calculations for the expected quantitative exposure scenario, which is only short-term inhalation exposure as described in the use

information section. However, in order to accomplish this a repeat dose NOAEL would be required, which was not available from the previous risk assessment.

For the purposes of this illustrative screening exercise, HED chose to use a repeat dose NOAEL of 0.02 mg/kg/day for the screening level assessment which is reflective of the low end of the previously used therapeutic doses, and is the same value that is used by the American Conference of Governmental Industrial Hygienists to derive their Threshold Limit Value (TLV) (ACGIH 2001). It should also be noted this value is used by the Occupational Safety and Health Administration https://www.osha.gov/dts/chemicalsampling/data/CH_268100.html) and National Institute for Occupational Safety and Health (http://www.cdc.gov/NIOSH/ershdb/EmergencyResponseCard_29750018.html) to derive their permissible and recommended exposure limits, respectively. This value is supported by the body of evidence of the literature and pharmaceutical research as well as the rigorousness of the ACGIH's process for selecting TLVs.

This NOAEL for this screening exercise is conservative for a number of reasons: 1) the data are based on human doses, yet the LOC (100, consisting of a 10X for intraspecies and a 10X for interspecies) used for the MOE screening was not reduced; 2) in the guideline acute lethality study with strychnine technical (MRID 40908901/41210701), there was no death observed at 1.4 mg/kg in females and 4.9 mg/kg in males (although it is unclear if clinical signs were observed at this dose); 3) no death or clinical signs of toxicity were observed in the acute oral study with the 1.8% formulation (MRID 44719701) at 50 mg/kg, a dose 4-fold less than the dose which caused death in females in the same study (200 mg/kg); 4) the NOAEL chosen is at the lower range of the human therapeutic doses; 5) toxicokinetic data indicate that strychnine is almost completely (90%) cleared from the body in one day, which is consistent with the observation that animals that survive in experimental studies stop displaying clinical signs between 1 and 24 hours after dosing; and 6) the NOAEL chosen is 100-fold lower than the acute dose that caused effects in the acute lethality study.

b. Developmental Rat and Rabbit Study

Prenatal development toxicity studies in both rats and rabbits are required in the 2007 revised 40 CFR Part 158 Toxicology Data Requirements for a non-food use chemical.

> *1. Evidence for developmental toxicity in the strychnine database:* Currently, there are no guideline developmental studies available for strychnine. There are two developmental studies in rats available in the literature (Boveyt-Nitti and Bovet 1959 and Garcia-Alcocer et al., 2005); however, both provide limited experimental data. In the more recent study, developmental effects were observed with treatment of strychnine, however they were seen at doses (2.5 mg/kg and higher by gavage) at or above the LD_{50} measured in the guideline acute lethality study (MRID 40908901/41210701) which limits the usefulness of these findings.
>
> *2. Evidence for developmental toxicity in the database of toxicology studies for other similar pesticides:* No structural analogues were available.

3. Risk Assessment Considerations: Strychnine is a non-food use chemical and no drinking water exposure is anticipated. Therefore, acute and chronic dietary assessments will not be conducted at this time. However, there is potential for *in utero* exposure to strychnine for pregnant occupational handlers by the inhalation route. However, developmental effects are not of a concern since: 1) developmental effects were only seen at doses that are acutely toxic; 2) the current endpoint based on the TLV is protective of the acute toxicity of strychnine and is considered conservative; and 3) exposure is expected to be limited due to the nature of the use pattern (limited application and underground use).

The HASPOC, based on a weight of the evidence approach, recommends that the developmental toxicity study in the rat and rabbit **not be required**, based on the following considerations: (1) the only evidence of developmental toxicity was seen at doses that are associated with acute toxicity in adults; (2) the current endpoint based off of the TLV is protective of the acute neurotoxicity of strychnine; (3) the limited exposure pattern for strychnine (limited application and underground use); and that a developmental study is not expected to result in a lower POD for risk assessment. .

c. Neurotoxicity Studies (Acute and Subchronic)

Acute and subchronic neurotoxicity studies are required in the 2007 revised 40 CFR Part 158 Toxicology Data Requirements, because they provide important scientific information on potential nervous system effects from pesticide exposure. These studies can provide data on a wide range of functional tests for evaluating neurotoxicity including sensory effects, neuromuscular effects, and histopathology of the nervous system. The HASPOC used a weight of evidence (WOE) approach to consider the need for acute and subchronic neurotoxicity studies to support the registered uses of strychnine.

1. Evidence for neurotoxicity in the strychnine database: Strychnine is a well-documented neurotoxicant and the central nervous system is the primary target organ for this chemical. Its mode of action involves antagonism of glycine receptors preventing the inhibitory function of this neurotransmitter. This leads to hyper excitability of motor neurons resulting in tremors, convulsions and eventually death due to asphyxiation. Thus, further neurotoxicity battery studies are not expected to provide useful information for the purpose of characterizing the toxicity of this chemical.

2. Evidence for neurotoxicity in the database of toxicology studies for other similar pesticides: No structural analogues were available.

3. Risk Assessment Considerations: Strychnine is a non-food use chemical and no significant drinking water exposure is anticipated. Therefore, acute and chronic dietary assessments will not be conducted at this time. Furthermore, while no repeat dose toxicity studies were available for strychnine, the required developmental study would provide information on this duration of exposure and therefore additional subchronic studies would not be useful for risk assessment.

The HASPOC, based on a weight of the evidence approach, recommends that the acute and subchronic neurotoxicity batteries not be required for strychnine, based on the following considerations: (1) the primary target organ is the central nervous system, which is already well characterized by the existing literature data; (2) no acute dietary risk assessment is needed since strychnine is a non-food use chemical and drinking water exposure is not anticipated.; and (3) additional studies addressing the neurotoxic effects of strychnine would not provide meaningful information for risk assessment purposes.

d. 90-Day Dermal Toxicity Study

A 90-day dermal study is required in the 2007 revised 40 CFR Part 158 Toxicology Data Requirements, based on the use pattern. For strychnine, dermal exposure is expected for both residential and occupational handlers. The HASPOC used a WOE approach to consider the need for a 90-day dermal study to support the registered uses of strychnine.

> *1. Indicators for Potential Dermal Toxicity:* The acute toxicity battery indicated that strychnine technical is highly toxic by the oral route (LD_{50} of 2.2 mg/kg in females, Category I) yet no toxicity was observed following acute dermal exposure up to a dose of 2000 mg/kg. Furthermore, no irritation, mortality, or signs of toxicity were observed in the dermal irritation study. The likely explanation is that the dermal absorption of strychnine is extremely low. This is supported by the NIOSH finite dose skin permeation calculator described above which estimates a dermal absorption value of 0.25% based on strychnine's physical chemical properties
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> *2. Chemical Structure:* Strychnine has a molecular weight of 334.4, low solubility in water (115 mg/L), and a log K_{ow} of 4.0 at pH 7.
>
> *3. Risk Assessment Considerations:* Based on the low dermal absorption and lack of acute toxicity following dermal exposure, despite potent acute toxicity following oral exposure, a quantitative occupational dermal risk assessment is not being conducted for strychnine.
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> *4. Evidence for Dermal Toxicity from SAR Chemicals:* No SAR Chemicals were available for comparison.

The HASPOC, based on a weight of the evidence approach, recommends that the 90 day dermal toxicity study **not be required for strychnine**, based on the following considerations: (1) no acute dermal toxicity was observed despite strychnine being a highly potent acute toxicant by the oral route; (2) the predicted dermal absorption of strychnine is well below 100%; and (3) a quantitative occupational dermal risk assessment is not being conducted for strychnine.

e. 2-generation Reproduction Study

A 2-generation reproduction study is required in the 2007 revised 40 CFR Part 158 Toxicology Data Requirements, if use of the product is likely to result in significant human exposure over a portion of the human life span in terms of frequency, magnitude or duration of exposure. For strychnine, significant human exposure is not expected across a human life span as the expected

exposure is short-term in nature. Furthermore, while the 2-generation reproduction study also provides useful information on direct exposure to the young and data in the literature in rats suggest that the young may be more susceptible to strychnine, no exposure to children is expected due to the underground use of strychnine and its overall limited use pattern. Therefore, a 2-generation reproduction study would not provide any additional useful toxicity information for the risk assessment based on the current use pattern.

The HASPOC, based on a weight of the evidence approach, recommends that the 2- generation reproduction toxicity study **not be required for strychnine** based on the following considerations: (1) significant human exposure over a portion of the human life span is not expected; (2) no exposure to children is anticipated from the current use pattern; and (3) the 2-generation reproduction study is not expected to provide a lower POD for risk assessment.

f. Subchronic Inhalation Study

An inhalation study is conditionally required in the 2007 revised 40 CFR Part 158 Toxicology Data Requirements for a non-food use chemical. However, based on the proposed use pattern there is potential for inhalation exposure to occupational and residential handlers. Previously, OPP used a set of criteria to determine whether or not an inhalation study could be waived. These criteria considered the scientific information available for the chemical, including its: 1) degree of irritation and corrosivity; 2) volatility; 3) aerosol particle size; and 4) Acute Toxicity Category and extrapolated MOEs (e.g., MOEs 10 times higher than the target). In 2009, OPP developed an issue paper on risk assessment approaches for semi-volatile pesticides. As part of that issue paper, an analytical comparison was conducted of oral and inhalation experimental toxicology studies. In general, this analysis showed that the degree to which oral PODs were protective of potential inhalation toxicity varied. In many cases the oral POD was protective, but in some cases the inhalation PODs were significantly more protective. Currently, OPP uses a WOE approach that builds upon OPP's experience using the criteria listed above and conclusions from the 2009 SAP. As approaches for route-to-route extrapolation continue to evolve and improve, OPP may incorporate additional considerations into the WOE analysis.

Inhalation exposure can be to vapors, droplets, and/or particles/dusts. The form of inhalation exposure is determined by a number of factors including physical-chemical properties, use pattern, and exposure scenarios. OPP's interim WOE approach considers:

1. *Physical-Chemical Properties:* Vapor pressure and Henry's law constant are key considerations with respect to volatilization after sprays have settled. Strychnine has a vapor pressure of 1.89×10^{-7} mm Hg at 25ºC. The Henry's law constant is 5.96×10^{-14} atm-m^3/mole at 25 ºC. However, low vapor pressure and/or Henry's law constant do not preclude exposure to aerosolized droplets or particles/dust.

2. *Use Pattern and Exposure Scenarios:* Any application scenario that leads to inhalation exposure to droplets needs to be considered in the WOE analysis for an inhalation toxicology study waiver request. HED acknowledges that typical agricultural application methods are more likely to lead to high occupational handler inhalation exposure by comparison, particularly to droplets, and may contribute to spray drift. However, for

strychnine, there are no exposure scenarios using such equipment. The highest inhalation exposure results from loading/applying granules by hand dispersal which is the basis for the discussion below.

3. *Margins of Exposure (MOEs):* The MOE estimates for inhalation scenarios were calculated using an oral toxicity study and should be considered in the WOE analysis for an inhalation toxicology study waiver request. In the past, OPP has used MOEs of approximately 10 times higher than the level of concern as a benchmark for granting waiver requests. The 2009 analysis suggests this approach is appropriate for most pesticides but not all. Using this interim WOE approach, MOEs from 10-100 times greater than the level of concern will be considered in combination with other factors discussed here. In the case of strychnine, multiple occupational handler scenarios have MOEs below 1000, including loading/applying granules by hand dispersal (230), spoon (880), and burrow builder (310) without a respirator. These MOEs were calculated with the conservative NOAEL of 0.02 mg/kg/day based on the TLV described in section IVa.

4. *Toxicological Effects:* The toxicological effects of strychnine were presented earlier in section III. Based on the limited data available, it does not appear that strychnine is more toxic by the inhalation route as compared to the oral route of exposure. For example, both an acute oral and inhalation study are available for the 1.8% formulation (no inhalation study was available for the technical). Deaths in female rats occurred at 200 mg/kg in the oral study and at 2.13 mg/L (~370.62 mg/kg) in the inhalation study. No effects were observed at 50 mg/kg in the oral study, while some clinical signs (ocular/nasal discharge, irregular respiration, hypoactivity, and hunched posture) were observed at 0.59 mg/L (~100 mg/kg) in the inhalation study.

Based on a WOE approach, considering all of the available hazard and exposure information, the HASPOC recommends that the subchronic inhalation toxicity study be required for strychnine. This approach considered all of the available hazard and exposure information for strychnine including: 1) the physiochemical properties for strychnine; 2) the hazard profile of strychnine; and 3) using the current estimated oral POD, there are several inhalation risk estimates that are of concern.

g. Immunotoxicity Study

1. *Evidence for potential immunotoxicity in the strychnine database of toxicology studies:* No indications of immunotoxicity were observed in the strychnine database or in the literature. Furthermore, strychnine is a neurotoxicant with a well-defined mode of action.

Table 1. Summary of Strychnine **Immunotoxicity Potential in the Available Toxicology Studies and literature**	
Parameter	**Findings**
Hematology Indicators (WBC changes)	None
Clinical Chemistry Indicators (A/G Ratio)	None
Organ Weight Indicators (Spleen/Thymus)	None
Histopathology Indicators (Spleen, Thymus, Lymph Nodes)	None
Toxicity Profile (Target Organs)	Central nervous system

2. *Evidence for potential immunotoxicity for structure activity relationship (SAR) chemicals:* No SAR chemicals were available for comparison.

3. *Risk assessment considerations:* Strychnine is a non-food use chemical and no significant drinking water exposure is anticipated. Therefore, acute and chronic dietary assessments will not be conducted at this time.

The HASPOC, based on a weight of the evidence approach, recommends that an immunotoxicity study **is not required** for strychnine at this time based on the following considerations: (1) there was no evidence for immunotoxicity in the database or literature; (2) strychnine is a known neurotoxicant with a well-defined mode of action; and (3) based on the current use profile no acute or chronic dietary assessments will be conducted at this time. Based on the toxicity profile and current use pattern, an immunotoxicity study is not expected to provide useful information for risk assessment.

h. Bacterial Reverse mutation assay, in vivo mammalian cell assay, in vitro cytogenetics.

Bacterial Reverse mutation assay, in vivo mammalian cell assay, in vitro cytogenetics studies are required in the 2007 revised 40 CFR Part 158 Toxicology Data Requirements. These studies have not been submitted for strychnine. The only available data on the mutagenicity of strychnine was an *in vitro* assay in the literature testing strychnine with two strains of s. typhimurium (Hoffman et al., 1987). The authors found that strychnine was a recombinagen at doses of 1.5 to 6 mM (~ equal to 500- 2000 mg/L). While recombinagenicity indicates induction of DNA damage, it is not sufficient alone to conclude that a compound can induce mutagenicity or clastogenicity. The authors did assay for mutagenicity under similar conditions and strychnine did not induce reversion of the hisG46, hisD3052, and hisC3076 alleles. The authors also cited two other papers in which strychnine was tested in *Drosophila* for mutagenicity and chromosomal aberrations. Both assays were negative. It is also important to note that the concentrations tested in the literature study were much greater than concentration in plasma of humans which is associated with lethality (~ 2.2 mg/L). Based on the potent acute toxicity of strychnine it is very unlikely that it would be mutagenic at doses lower than those that result in significant toxicity or death.

The HASPOC, based on a weight of the evidence approach, recommends that bacterial reverse mutation assay, in vivo mammalian cell assay, and in vitro cytogenetics studies **not be required** for strychnine based on: (1) the lack of mutagenicity in the literature studies; and (2) the potent acute toxicity of strychnine.

V. HASPOC CONCLUSIONS

The HASPOC, based on a weight of the evidence approach, concludes that the 90-day dermal study, prenatal developmental study in rat, prenatal developmental study in rabbit, 2-generation reproduction and fertility effects study, bacterial reverse mutation assay, i*n vitro* mammalian cell assay, i*n vivo* cytogenetics assay, acute neurotoxicity study, subchronic neurotoxicity study, and the immunotoxicity study not be required for strychnine.

The HASPOC concludes, based on a WOE approach, that a subchronic inhalation toxicity study is required for strychnine. In the absence of this study, a 10X database uncertainty factor will be applied only for assessing risk for inhalation scenarios until an inhalation study is submitted or other information is provided to support a waiver. Due to the high acute toxicity of the compound and the fact that the primary effect of the chemical is neurotoxicity, a guideline 90-day study is not recommended. Instead, a subacute inhalation study that is well designed to identify the key neurotoxic effects (clinical signs) at the critical time of peak effect, in addition to the typical evaluations of a guideline inhalation study, is recommended. Because this would be a non-guideline study, the registrant is encouraged to contact the Agency for guidance on designing the protocol prior to conducting the study. Based on the inhalation exposure/risk profile for strychnine, the addition of a respirator that offers 80% reduction (i.e., PF5) in exposure (e.g., a filtering facepiece) to registered labels as personal protective equipment for certain scenarios would result in inhalation MOEs above 1000; in which case an inhalation study would not be required, pending finalization of modified labels.

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